10% emulsion of rabbit brain which was free of virus. All animals survived an observation period of 14 d without any signs of disease.

S. BANIC

Institute of Microbiology,
Medical Faculty,
61105 Ljubljana, Yugoslavia

Received July 17; accepted October 1, 1975.

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Increased insulin binding capacity of liver membranes from diabetic Chinese hamsters

THE first evidence for an abnormality in insulin receptor capacity in pathological states was provided by Kahn *et al.*[1], who found that liver plasma membranes from the obese hyperglycaemic mouse bound only a fraction of insulin compared with membranes from lean littermates. A similar observation was made with lymphocytes from obese insulin-resistant diabetic patients[2]. In lymphocyte cultures the insulin concentration in the medium seemed to determine the binding capacity of the cells[3]. These results suggested an inverse relationship between the number of receptors and the surrounding concentration of insulin, regardless of the tissue sensitivity to the hormone. We have now found greater than normal binding capacity in the diabetic Chinese hamster, which has genetic insulin deficiency, in many ways resembling juvenile diabetes[4].

Table 1 Characteristics of control and diabetic groups and the respective plasma membrane preparations (10 animals in each group)

	Control	Diabetic
Body weight (g)	36.9±0.3	34.8±0.8
Liver weight (mg)	1.45±0.05	1.71±0.04
Blood sugar (mg per 100 ml)	130±6	468±59
5′-Nucleotidase (μmol mg^{-1}×min)	0.36	0.33
Mg^{2+}-ATPase (nmol mg^{-1}×min)	179.2	168.8
Recovery of membrane protein (mg per g wet weight)	1.03	0.94

5′-Nucleotidase was measured as recommended by Solyom *et al.*[6] Mg^{2+}-ATPase was assayed by measuring the release of ^{32}P-orthophosphate from γ-^{32}P-ATP (ref. 7).

Plasma membranes were prepared by the method of Neville[5] from diabetic Chinese hamsters aged 4–5 months and from control animals of comparable age and weight (Table 1). ^{125}I-insulin binding was assayed after incubation for 20 min at 25 °C; membrane-bound hormone was separated from free hormone by centrifugation through silicon oil (W.K., R.R., A. Zynamon and K.D.H., in preparation). Figure 1 is a Scatchard plot from a representative experiment. As previously observed in rats, the points fit two curves corresponding to high affinity–low capacity (site I) and low affinity–high capacity (site II) binding sites[8]. The constants derived from this figure are—for site I: control, 4.2×10^{-13}, diabetic, 9.0×10^{-13} mol per mg protein; for site II: control, 1.9×10^{-12}, diabetic, 4.0×10^{-12} mol per mg protein. The corresponding affinity constants are approximately 10^{-9} for site I, and 10^{-8} mol l^{-1} for site II, in both groups. Recovery of membrane protein and enzyme markers was similar for both groups (Table 1). Figure 1 shows that the binding capacity for both classes of binding sites (intercept at abscissa) per mg protein is considerably higher in the diabetic animals, whereas there is no apparent change in affinity (represented by the slope). Although other interpretations of nonlinear Scatchard plots, such as negative cooperativity[9], polymerisation of the hormone[10] or heterogeneity of the labelled hormone[11] are possible, the essential observation—of an abnormally high binding capacity in membranes from diabetic hamster—remains the same.

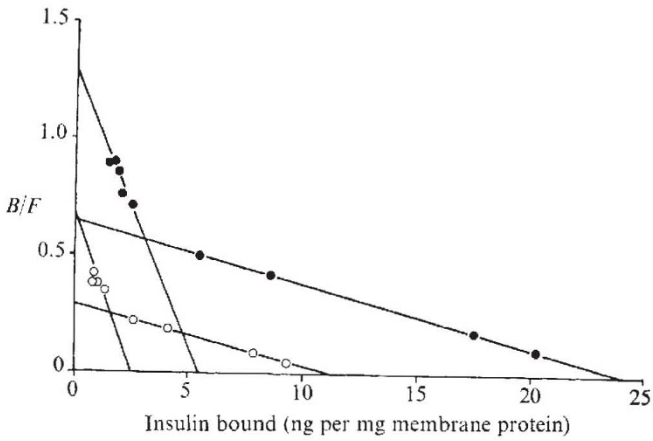

Fig. 1 Scatchard plot of insulin–membrane interaction in two representative preparations from 10 control (○) and 10 diabetic (●) animals. Incubation was performed at 25 °C for 20 min with 0.2 mg of membrane protein per ml and insulin concentrations between 4 and 400 ng ml^{-1}. Insulin degradation was 1.3% in these conditions. Nonspecific binding, that is labelled hormone not displaced by 1 U ml^{-1} has been subtracted.

Severely diabetic Chinese hamsters have abnormally low plasma and pancreatic insulin, a low insulin response to a glucose load and, consequently, low glucose tolerance[4]. Together with a tendency for ketosis[4], these features resemble juvenile diabetes in man, and the diabetic Chinese hamster provides an interesting model for this type of the disease. Apparently the liver adapts to the decreased hormone concentration by raising the number of receptors in the plasma membrane, whereas, conversely, in the hyperinsulinaemic C57BL ob/ob mouse[1] as well as in the KK-mouse[12], the total receptor capacity is lower than normal. Our data therefore support the hypothesis that the hormone level controls the number of receptor sites in the liver cell.

We thank Dr Karl Thomae, GmbH Biberach for diabetic and normal hamsters.

K. D. HEPP
JANE LANGLEY
H. J. VON FUNCKE
R. RENNER
W. KEMMLER

Institute for Diabetes Research and 3rd Medical Department,
Schwabing City Hospital,
8 Munich 40, Kölner Platz 1,
West Germany

Received August 11; accepted September 24, 1975.

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